

CLAIMS

1. A method of producing a DNA library comprising a plurality of DNA sequences of interest, each DNA sequence of interest having at least two predetermined positions, with at each predetermined position a codon selected from a defined group for that position, the codons within a group coding for different amino acids, said method comprising the steps of: -
 - (i) contacting so as to effect hybridisation (a) template DNA comprising said at least two predetermined positions, said template DNA being fully randomised at said at least two predetermined positions, (b) for each predetermined position, a selection oligonucleotide pool, each selection oligonucleotide within each pool comprising a codon selected from the defined group for that predetermined position, and (c) at least one additional oligonucleotide sequence comprising a region which is non-hybridisable to the template DNA,
 - (ii) ligating the hybridised DNA sequences,
 - (iii) denaturing the product of step (ii) so as to give a mixed population of said template DNA and said DNA sequences of interest, and
 - (iv) selectively amplifying the DNA sequences of interest,wherein said additional oligonucleotide sequence of step (i) is selected such that after step (ii) the non-hybridisable region is located externally of the template DNA.
2. The method of claim 1, wherein the defined group consists of the MAX codons which represent the optimum codon usage in a predetermined organism of interest, or a predetermined selection of said MAX codons.
3. The method of claim 1 or 2, wherein the defined group consists of the codons AAA, AAC, ACC, AGC, ATG, ATT, CAG, CAT, CCG, CGC, CTG, GAA, GAT,

- 26 -

GCG, GGC, GTG, TAT, TGG, TGC, TTT which represent the MAX codons in the model organism *Escherichia coli*, or a predetermined selection therefrom.

4. The method of claim 2 or 3, wherein one or more of the MAX codons is substituted for an alternative codon coding for the same amino acid.
5. The method of any preceding claim, wherein the defined group consists of codons which code for amino acids having similar properties.
6. The method of claim 5, wherein said similar properties may be acidity or basicity, and/or hydrophobicity or hydrophilicity, and/or polarity or non-polarity.
7. The method of any preceding claim, wherein the defined group for each position is independently selected.
8. The method of any preceding claim, wherein the additional oligonucleotide sequence forms part of the oligonucleotides in one of the selection pools.
9. The method of any one of claims 1 to 7, wherein the additional oligonucleotide sequence is a separate oligonucleotide having a region complementary to the 5' end of the template DNA.
10. The method of any preceding claim, wherein in step (i) each selection oligonucleotide pool is added in excess of useable template DNA.
11. The method of claim 10, wherein the ratio of each selection oligonucleotide pool to useable template DNA is at least 2:1, preferably at least 5:1, more preferably at least 10:1, and most preferably about 12:1.

12. The method of any preceding claim, wherein, the template DNA is attached to a support prior to step (i) such that after the denaturation of the double stranded DNA construct formed in step (ii), the template DNA is removed before step (iv), step (iv) being effected by PCR utilising the overhanging non-hybridisable region of the additional oligonucleotide sequence as a primer binding site.

13. The method of any one of claims 1 to 11, which includes a step of contacting a second additional oligonucleotide sequence in step (i), said second additional oligonucleotide also comprising a non-hybridisable region, the second additional sequence being designed such that after step (ii) it is located at the 5' end of the sequence of interest, with the non-hybridisable region overhanging the 3' end of the template DNA, and wherein step (iv) is effected using first primer complementary to the non-hybridisable region of the first additional sequence, and a second primer identical to the non-hybridisable region of the second additional sequence.

14. The method of claim 13, wherein the second additional sequence forms part of the oligonucleotides in one of the selection pools

15. The method of any preceding claim, wherein the amplified DNA sequences of interest are inserted after step (iv) into a suitable cloning vector.

16. The method of claim 15, wherein the cloning vector is a prokaryotic or eukaryotic expression vector, an integrating vector or a bacteriophage vector, chosen according to the intended use of the library.

17. The method of claim 14 or 15, wherein prior to insertion into the cloning vector, the DNA sequences are digested by a restriction endonuclease in order to

- 28 -

generate the required cassette for cloning, a restriction endonuclease recognition site being present in the required location in the sequences of interest.

18. The method of claim 17, wherein the recognition site is provided in the initial template DNA.

19. The method of any preceding claim, wherein the sequences of interest are inserted into an appropriate gene.

20. A DNA library producible by the method of any one of claims 1 to 19.

21. A method of producing a protein library comprising a plurality of polypeptides, each polypeptide having a different combination of amino acid residues in at least two predetermined positions, said method comprising the step of expressing the sequences of interest produced by the method of any one of claims 1 to 19 or from the DNA library of claim 20.

22. A protein library producible by the method of claim 21.

23. The use of the protein library of claim 22 to investigate binding interactions between the proteins (polypeptides) in the library and any appropriate ligand such as DNA, and other proteins or ligands.

24. The use of claim 23, to investigate the binding interactions of randomised zinc fingers or randomised antibodies.